Saskatchewan
Agriculture
and Food

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20030533

DEVELOPMENT OF MOLECULAR MARKERS FOR BETA-GLUCAN CONTENT IN BARLEY

Funded by: The Agriculture Development Fund

March 2008

Prepared by: University of Saskatchewan (U of S)

Jan 08

Final Report

April 1, 2004 – Dec 31, 2007

Saskatchewan Agriculture Development Fund Project # 20030533

Development of molecular markers for beta-glucan content in barley

Principal Investigator – Dr. Ravindra Chibbar

Co-investigators: Dr. Monica Båga and Dr. Brian Rossnagel

Department of Plant Sciences and Crop Development Centre

College of Agriculture and Bioresources, University of Saskatchewan

Saskatoon, Saskatchewan

Summary for website (100 words): Beta-glucan is a complex polysaccharide found in the barley endosperm. Barley with low beta-glucan is preferred both by the brewing and animal feed industry. However for human consumption barley with high beta-glucan is preferred because of its health benefits. The objective of this project was to identify barley chromosomal regions associated with beta-glucan concentration. A genetic map (1,059 cM) was constructed with 680 DNA-based markers distributed over the seven chromosomes. A region on chromosome 7 was associated with beta-glucan concentration. The barley genetic map developed in this project can be used for other traits of interest to barley improvement programs.

Abstract / Summary: The amount of (1→3),(1→4)-*β*-D glucan (*β*-glucan) accumulated in cell walls of barley (*Hordeum vulgare* L.) grain is an important determinant for the grain end-use. Initially, *β*-glucan concentration was determined using a colorimetric method (Megazyme) and flow injection method. Twenty one malting/feed barley cultivars/breeding lines were initially screened for beta-glucan concentration using both the methods. The colorimetric (Megazyme) method in general gave higher beta-glucan concentrations as compared to the flow method. However, trends in the beta-glucan concentrations were similar using both the methods. Four barley lines, TR253, TR251, CDC Bold and Valier were selected for detailed structural analysis in collaboration with Dr. Marta Izydorczyk, Grain Research Laboratory Winnipeg, Manitoba.

The *β*-glucan concentration in grain is affected by environment and genetic factors and was studied in a doubled-haploid (DH) population of 170 lines grown in three separate field trials. Most of the DH lines showed *β*-glucan values that ranged from that of the low *β*-glucan parent (cultivar CDC Bold; ~3.3%) to that of the high *β*-glucan parent (breeding line TR251; ~5.4%) Eighty-eight lines of the DH population were genotyped using simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and diversity array technology (DArT) markers, which were subsequently integrated into barley genetic map spanning 1,059 cM. Single-marker analysis of phenotypic data from the three trials indicated ten genomic regions that were all associated with low *β*-glucan concentration in grain. For all putative QTLs, the trait was favored by alleles from CDC Bold. A dominant QTL located to the centromere region of chromosome 7H was identified by interval mapping for all three trials. The 7H QTL explained up to 55.7% of the *β*-glucan concentration and genetic markers associated with the locus may be used to aid selection of high and low *β*-glucan barley lines.

Introduction and Background:

The major storage components in cereal grains are carbohydrates, of which water-insoluble starch is the predominant form in the endosperm. The less abundant complex

carbohydrates, e.g. arabinoxylans and β-glucans are concentrated in cell walls and constitute a substantial amount of soluble dietary fiber in cereal grains. In barley about 70% of total soluble dietary fiber is derived from β-glucan molecules (Fincher 1975).

β-glucan is a linear polymer made up by 3-D-glucopyranosyl molecules that can form β-1,4- and β-1,3 linkages. The β-1,4- linked molecules constitute primarily cellotriose and cellotetraose units (90-95%), which are joined by β-1,3 linkages in a ratio of about 2.5:1 (Buckeridge et al., 2001). The amount of β-glucan in the kernel is one of the determining factors for the end-use of the grain. In the malting industry, a grain with low β-glucan concentration is preferred, as the breakdown β-glucans increases the viscosity of wort, which negatively affects brewing efficiency and beer filtration rates (Stewart et al., 1998). Higher viscosity is also obtained upon digestion of β-glucan molecules in animals and humans leading to lower absorption of nutrients in the digestive tract. The low nutritious value associated with β-glucan-rich grain is a problem for the monogastric animal feed industry, but for human food, the reduced uptake of nutrients in the small intestine is considered beneficial to health. Lower postprandial blood sugar (Cavallero et al., 2002) and serum LDL-cholesterol (Hecker et al., 1998) levels are associated with β-glucan-rich diets, which therefore may reduce the risk of coronary heart disease and type II diabetes (Poppitt et al., 2007).

Like most grain storage components, the amount of β-glucan deposited during grain filling is affected by the environment and multiple genetic factors which contribute to the quantitative trait (see reviews by Fox et al., 2003 and Zale et al., 2000). The study of quantitative traits in crops has rapidly evolved during the last decade, due to the emergence of molecular markers, which have become increasingly useful tools for accelerating crop improvement. In barley, a large number of restriction fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence tagged sites (STS), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers are available for mapping (http://wheat.pw.usda.gov). In addition, a recently developed high-throughput and low cost diversity array technology (DArT®) based on DNA hybridization, is applicable to whole genome scanning in barley (Wenzl et al., 2004 and 2006). Genotyping of various barley mapping populations using genetic markers has led to construction of 71 genetic linkage maps and six consensus genetic maps with increasingly higher precision over the years (reviewed by Backes et al., 2006).

The grain β-glucan concentration is often included in the malting quality evaluation of barley. Malting quality is a complex characteristic, which encompasses malt extract percentage, total and soluble protein content, soluble/total protein ratio, kernel plumpness, α- and β-amylase activities, diastatic power, dormancy, and malt β-glucanase activity in addition to β-glucan concentration in grain and malt. The first QTLs for malting quality were identified for α-amylase genes on chromosomes 2H, 4H and 6H (Hayes et al., 1993). By the year 2000, a total of 168 QTLs for 19 malting quality traits had been identified among nine populations (Zale et al., 2000). All the barley seven chromosomes are associated with the β-glucan concentration in grain or malt (Han et al., 1995; Mather et al., 1997; Ayoub et al., 2002; Gao et al., 2004; Molina-Cano et al., 2007), but the individual QTL effects may be affected by environmental conditions (Molina-Cano et al., 2007).

The β-glucan concentration in barley grain normally ranges from three to six percent (Izydorczyk et al., 2000), but mutant lines with concentrations up to 19 % have been reported (Seefeldt et al., 2007). Low starch barley lines, such as high amylose or waxy barley often show β-glucan concentrations above 6% (Izydorczyk et al., 2000), which may indicate a partial

reallocation of photosynthate products from starch biosynthesis to cell wall polysaccharides in these mutants. To avoid the confounding effects of starch mutations on β-glucan biosynthesis, we have for this study chosen barley lines that show normal starch characteristics, but differ in β-glucan concentration. The DH mapping population used was derived from a cross between CDC Bold (~3.3% β-glucan) and breeding line TR251 (~5.4% β-glucan) and derived DH lines showed a 2.5% range in β- glucan concentration. Three field trials were conducted for collection of phenotypic data and genomic regions associated with low β-glucan concentration were identified by QTL mapping.

Two cell wall genes, cellulose synthase *CesA1* and *CesA2*, were first isolated by random sequencing of cotton expressed sequence tags (Pear et al. 1996). Based on the complete genomic sequence of *Arabidopsis* and rice *CesA* genes and cellulose synthase like (*Csl*) genes family was reported and identified in maize and in some other plants (Holland et al. 2000; Tanaka et al. 2003; http://cellwall.stanford.edu). The barley *CesA* gene family was not found to be associated with any phenotypic or QTLs related to glucan synthase mapped on seven chromosomes (Burton et al. 2004 and 2005). *CslA*, *CslC*, *CslD*, and *CslE* are common gene families in *Arabidopsis* and rice. Rice lacks the *CslG* and *CslB* families and possesses two additional, *CslF* and *CslH*. Burton et al. (2006) used comparative mapping to link a major QTL of β-glucan concentration in barley to a rice syntenic region which contained a *CslF* gene cluster. Transgenic expression of the identified *CslF* gene in Arabidopsis resulted in the identification of (1,3;1,4)-β-glucan. This suggested that *CslF* genes can be potential candidate genes to mediate (1,3;1,4)-β-glucan biosynthesis in grasses and cereals. Further study on biochemical functions of this gene product will help us to understand the structure function of *Csl* protein and differences in cell wall architecture between monocots and dicots (Young et al. 2007). Therefore, the objectives of this research were:

- Identify molecular markers tightly linked to high and low β-glucan content in the barley grain.
- Use barley populations as model systems for optimization of genetic fingerprinting methods for crops.
- Make identified markers and procedures used for screening lines available for development of malting and feed barley with low β-glucan content.

Material and Methods:

Barley genotypes and determination of grain β-glucan concentration

A barley doubled-haploid (DH) population of 243 lines was developed at Agriculture and Agri-Food Canada (AAFC), Brandon Research Centre, Brandon, Manitoba, Canada, from a cross between a moderately high β-glucan parent, two-rowed breeding line TR251 (developed at AAFC, Brandon), and a low β-glucan parent, two-rowed semi-dwarf cultivar, CDC Bold. The DH population of 170 lines and parents were planted in an un-replicated randomized block design with repeated checks in New Zealand (2004) and Saskatoon, Canada (2005 and 2006). The total β-glucan concentration in harvested grains was determined from fifteen kernels that were cut in halves and ground into a fine powder using a WIGL BUG grinding machine improvised in our laboratory. Three samples of 50 mg of flour each were used for extraction of total β-glucans, which were analyzed by the calcofluor flow injection method (Astrup and Jorgensen, 1988; Switala et al. 1989) using a spectrofluorometer for quantifications. The amount of total β-glucan was calculated from a standard curve established using a commercial β-glucan

standard (Megazyme International Ireland, Ltd.) and values were expressed as percent β-glucan in dry grain. One-way ANOVA was performed to test the significance of differences between the genotypes and the three field trials.

Genetic mapping

The parents and 88 DH lines were planted in soil in growth chambers maintained at 20°C in 16-h light at 320 μmol m^{-2} s^{-1}. Leaf samples were harvested at five to ten leaves stage, frozen in liquid N_2, and stored at -80°C. DNA was extracted from the leaf tissues using a CTAB method and quantified by UV spectrophotometry.

A total of 194 microsatellite markers (Liu et al. 1996; Ramsay et al. 2000) were tested for polymorphisms between the parents TR251 and CDC Bold. The PCR reactions consisted of 100 ng template DNA, 5 pmol of each primer , 10 mM Tris-HCl pH 8.3, 3.5 mM $MgCl_2$, 25 mM KCl, 20 mM dNTP, 0.5 U of Red Taq Polymerase (Sigma) in a total volume of 12.5 μl. Amplifications were done in a MyCycler thermal cycler (Bio-Rad Lab. Hercules, CA, USA) programmed for an initial denaturation at 94°C for 5 min followed by 32 cycles of 45 s denaturation at 94°C, 20 s annealing at optimized temperature and 90 s extension at 72°C. In the final cycle, the elongation step at 72°C was extended by 10 min. A 2.5 μl aliquot of the PCR reaction products was mixed with 2.5 μl 2 × concentrated loading buffer (95% w/v formamide, 10 mM NaOH, 0.05% w/v bromophenol blue, and 0.05% w/v xyanol blue) and denatured at 95°C for 2 min. Samples of 3.0 μl were loaded onto 6% w/v denaturing polyacrylamide gels, and DNA fragments were separated using a Gibco BRL S2 sequencing apparatus. Silver staining following the Silver Sequence protocol (Promega, Madison, WI, USA) was performed to visualize separated DNA fragments. When the size difference between polymorphic alleles was >10 bp, the PCR products were separated by 2% w/v agarose gel electrophoresis and fragments were visualized by ethidium bromide staining.

Amplified fragment length polymorphism (AFLP) analysis was done essentially as described by Vos et al. (1995) using 64 EcoRI/MseI primer combinations. Separation of AFLP fragments, scoring and size determinations were as described (Bâga et al. 2007).

The genotype data using Diversity Arrays Technology (DArT) markers (Wenzl et al. 2004, 2006) were generated by Triticarte Pty Ltd (Canberra, Australia; www.triticarte.com.au), as described (Wenzl et al. 2006). Total DNA extracted from leaves of 88 DH lines and the two parent lines was used in the analysis.

Map construction and QTL analysis

The Joinmap3 software (van Ooijen and Voorrips, 2001) was used to construct a genetic map of barley. Markers were assembled into linkage groups at likelihood ratio statistic (LOD) ≥6.0 and assigned to the seven chromosomes of barley based on published data for microsatellite and DArT markers (Ramsay et al. 2000; Wenzl et al. 2006). Recombination frequencies were converted into centiMorgan (cM) map distances using the Kosambi mapping function (Kosambi 1943). Segregation ratios for each locus and deviations from expected ratios were determined using the chi-square (χ^2) test.

The association between each single marker and phenotype was assessed using the Qgene 3.0 software (Nelson 1997). Putative QTLs were assigned for markers when the following criteria were met: a significant effect was observed for single marker/trait combination with $p<0.001$ in one investigation; significant effects in the same direction were observed for a single/trait combination with $p<0.01$ in two or more investigations; significant effects in the

same direction were observed for a single maker/trait combination with $p<0.1$ in three assessments (Fulton et al. 1997). The percent phenotypic variance associated with each significant QTL was calculated from regression analysis of each maker/phenotype combination using the Qgene software.

The MapQTL5 software (van Ooijen 2004) was used to identify QTLs by interval mapping. The significant genome-wide LOD threshold with a p-value of 0.05 was determined by 1,000 permutation tests (Churchill and Doerge 1994).

Identification of putative candidate genes:

Using comparative analysis of barley ESTs and rice sequences, 49 primer pairs were designed for 18 cellulose synthase (*CesA*) and 31 *Csl* genes used to identify putative candidate genes. Two malt beta-glucan QTLs identified in a region between two RFLPs *Bhm1B* and *Amy2* in Steptoe × Morex population (Han et al. 2004). Five primer pairs were gained from these two genes (Han et al. 1997; Knox et al. 1987).

Single Strand Conformation Polymorphism (SSCP) gel screening

PCR products with a size range of 100-600 bp were separated with 10% polyacrylamide gel using Bio-Rad protean II running apparatus. Each gel solution was prepared with 20ml 25% Acrylamide:bis (49:1), 6ml 5 × TBE buffer, 5ml 50% glycerol, and 19ml H_2O. After degassing for 5 minutes, 50ml gel solution was added 35µl of TMED and 165µl 10%APS. A comb of 1.5mm was inserted carefully and 20 wells would be available after 1 hour. The 10% Acrylamide was polymerized then removed gel sealing tape if used in case the gel solution leaking from two glass plates.

A mixture of 8µl PCR product and 6µl 0.1% bromophenol blue and xylene was denatured with heating at 95°C for 10 minutes and immediately kept on ice bath, then, 10µl loaded to the gel. The buffer chamber was filled with 0.6 × TBE buffer. Before loading, the gel wells were rinsed to remove unwanted particles and any excess acrylamide. Running temperature 17°C was maintained using a thermostatically controlled refrigerated circulator and running time was around 17-20 hours depending on the size of PCR products. Electrophoresis conditions were set on 5W (power-constant), 300V (voltage), and 50A (current), respectively.

Each gel was washed with fix solution (10%EtOH and 0.5% Acetic acid) for 3 minutes, and stained with staining solution (10%EtOH, 0.5% Acetic acid, and 0.2% $AgNO_3$) for 5 minutes. Then, the gel was rinsed with water for 10 seconds and developed with developing solution (3% NaOH and 0.1% Formaldehyde). The whole process of washing, staining and developing was carried out with gentle shaking. Bands stained were visualized then stopped development and washed with water. Finally, the gel was kept in water and photographed for screening.

Results:

Analysis of β-glucan concentration in grain

Analysis of beta-glucan concentration in selected NIL, DH and parent lines: We have used two methods to screen parental lines to select material for detailed molecular mapping work (Table 1).

CDC Bold in all the three determinations showed relatively low beta-glucan concentrations as compared to other lines studied. Valier, CDC Dolly, TR251, TR 253, Harrington and a breeding line 16092 showed relatively higher beta-glucan concentrations.

Although the absolute concentrations varied between the three methods, but the trends were similar in all the three determinations, two conducted at Saskatoon and one at Grain Research Laboratory Winnipeg. Based on these results, we decided to select CDC Bold × TR251 and a DH population developed from this cross, for detailed molecular mapping analysis. Valier and TR253 were also selected as high beta-glucan lines for confirmatory studies. The very high β-glucan concentration of Valier was as experimental aberration.

Table 1 β-glucan concentration in selected barley cultivars and breeding lines.

No	Cultivar/ Breeding Line	β-glucan concentration (%) / determination method		
		Concentration/Megazyme (Saskatoon)	Concentration/Flow-Injection (Saskatoon)	Concentration/Megazyme (GRL Winnipeg)*
1	Derkado	2.92 ± 0.24	3.68	
2	B83/12/21/5	3.14 ± 0.05	3.56	
3	CI4196	4.35 ± 0.40	4.18	
4	TR244	4.51 ± 0.13	4.10	
5	TR253	6.39 ± 0.07	4.34	4.15 ± 0.02
6	TR251	6.50 ± 0.10	4.49	5.13 ± 0.01
7	TR361	4.65 ± 0.07	3.77	
8	2nd 16092	6.61 ± 0.04	4.44	
9	Harrington	7.17 ± 0.12	4.64	
10	Manley	5.25 ± 0.22	4.00	
11	Valier	9.85 ± 0.12	5.33	4.27 ± 0.00
12	HDE84 194-622-1	5.14 ± 0.04	3.90	
13	TR306	4.42 ± 0.13	3.94	
14	CDC Bold	2.19 ± 0.03	2.94	3.47 ± 0.01
15	RFLP Harrington	5.94 ± 0.12	4.44	
16	CDC Dolly	6.81 ± 0.08	4.68	
17	CDC Helgason	4.76 ± 0.06	4.14	
18	Arapiles	5.06 ± 0.07	4.16	
19	Franklin	5.20 ± 0.27	4.26	
20	CDC Dolly	6.67 ± 0.07	4.79	
21	Beta Glucan Std Flour	4.41 ± 0.02**	3.72	

**Expected value = 4.4

The β-glucan concentration in grains of the TR251, CDC Bold and 170 DH lines was evaluated in three consecutive field trials conducted in New Zealand (2004), Saskatoon (2005 and 2006). The β-glucan concentration in TR251 (4.3-5.2%) was consistently higher than in CDC Bold (3.3-3.8%) for all three trials (Table 2), whereas the β-glucan concentrations among the DH lines showed a continuous distribution (Figure 1), which is typical for a quantitative trait. One-way ANOVA analysis also revealed a significant difference identified for the trait ($p<0.001$) gained in each year for each line of the population. Most of the DH lines showed β-glucan values that were between those of the parents in each year, but transgressive segregation was noted for 15 DH lines with low β-glucan values. Highly significant differences ($p<0.001$) were noted for β-glucan concentrations in the three field trials, suggesting that environmental differences influenced the amount of β-glucan accumulated in the grain. As compared to field trials in 2004 and 2006, the β-glucan concentrations for the DH lines in 2005 were overall lower and showed a more narrow distribution (Figure 1; Table 2). Nevertheless, the trait data displayed significant differences between the DH lines in each year to allow QTL analysis of trait data.

Table 2. Distribution of grain β-glucan concentration in parent and DH lines.

Year	Location	Parents		DH population		
		CDC Bold (%)	TR251 (%)	Range (%)	Average (%)	Standard deviation
2004	New Zealand	3.8	5.2	2.5 - 5.1	4.2	0.42
2005	Saskatoon, Canada	3.3	4.3	2.9 - 4.6	3.7	0.37
2006	Saskatoon, Canada	3.8	4.6	3.3 - 5.7	4.3	0.46

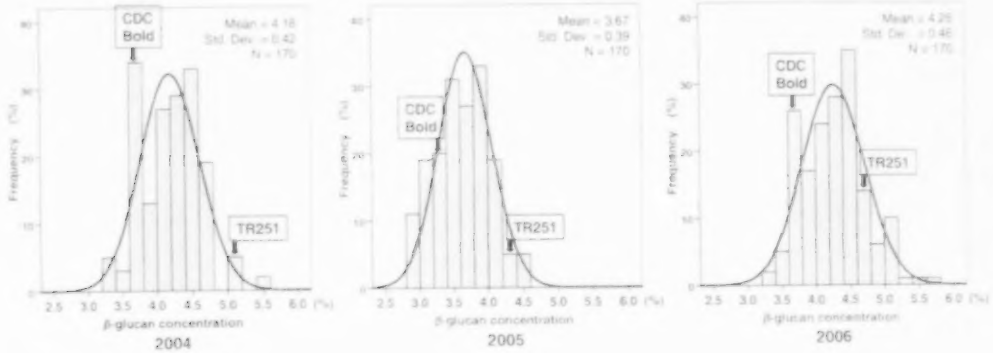


Figure 1. Distribution of grain β-glucan concentration for three field trials.

Histogram of β-glucan concentration in 170 barley DH lines grown in New Zealand in 2004, Saskatoon in 2005 and Saskatoon in 2006. Level of β-glucan concentration measured for parent lines, CDC Bold and TR251, grown in the trials is indicated.

Map construction and marker segregation

A sub-set of 88 DH lines from the TR251 × CDC Bold population was selected to represent the complete population based on the grain β-glucan concentrations determined from the New Zealand trial in 2004 (Figure 1). The 88 DH lines were initially genotyped using SSR markers, but screening of parent lines, TR251 and CDC Bold, revealed a quite low level of polymorphism. Among 194 SSR markers screened, a total of 66 SSR markers (34%; 73 alleles) could be used for mapping of the DH population. To increase the genotype data, the mapping population was subjected to AFLP analysis using a total of 64 different *EcoR*I+3 / *Mse*I+3 primer combinations, which produced 539 polymorphic loci (Table 3). An additional 452 loci were mapped using DArT markers. In total, 861 scored loci were obtained for the 88 DH lines, and after exclusion of singletons and co-segregating markers, a total of 680 loci were mapped (Table 4). The 680 loci, including 378 AFLP, 243 DArT, and 59 SSR loci, could be grouped into eight linkage groups which were assigned to seven chromosomes based on alignments to previously published maps (Liu et al. 1996; Ramsay et al. 2000; Wenzl et al. 2006). Chromosomes 1H, 3H, 4H, 5H, 6H and 7H were each represented by a single linkage group, whereas the short and long arm of chromosome 2H was split onto the two remaining linkage groups (Figure 2). The distribution of markers on chromosomes, 1H, 2H, 3H, 5H and 6H was relatively even with 87 to 106 markers assigned to each chromosome. Chromosome 7H was over represented with markers (180) and chromosome 4H showed a low level of polymorphism which

resulted in only 22 loci being mapped. Similar to previously published barley maps, the distribution of markers along the chromosomes was characterized by regions with tight clustering of markers and regions with low marker density. The length of the chromosomes varied from 60 cM (4H) to 207 cM (5H) and the total distance of the genetic map was 1,059 cM with an average marker density of 1.56/cM (Figure 2).

Table 3 Summary of polymorphism in 64 AFLP primer combinations

		M47	M48	M49	M50	M59	M60	M61	M62	Total
		MU+CAA	MU+CAC	MU+CAG	MU+CAT	MU+CTA	MU+CTC	MU+CTG	MU+CTT	
E32	EU+AAC	11	7	10	8	4	8	3	7	**58**
E33	EU+AAG	10	6	7	8	15	15	10	7	**78**
E35	EU+ACA	13	10	5	18	11	7	12	6	**82**
E36	EU+ACC	13	7	3	7	11	6	2	6	**55**
E37	EU+ACG	11	8	4	7	6	11	8	13	**68**
E38	EU+ACT	21	9	8	10	12	12	7	15	**94**
E40	EU+AGC	9	11	5	8	4	7	2	8	**54**
E41	EU+AGG	8	4	6	9	9	4	5	5	**50**
Total										539

Table 4. Distribution of AFLP, DArT and SSR markers on barley chromosomes.

Chromosome	Length (cM)	AFLPs	DArTs	SSRs	Sum
1H	158	45	32	10	87
2H	98/81	47	45	6	98
3H	178	54	42	5	101
4H	61	7	8	7	22
5H	207	50	33	5	88
6H	122	61	38	7	106
7H	154	114	45	19	178
Total	1059	378	243	59	680

Of the 680 loci mapped to seven barley chromosomes, 283 (42%) and 102 (15%) marker alleles deviated significantly from the expected 1:1 segregation ratio at $p< 0.05$ and $p< 0.001$, respectively. 163 loci on chromosomes 2H, 5H, 6H and 7H skewed toward CDC Bold alleles and 120 markers favoring TR251 alleles were identified on all chromosomes except 6H (Figure 2).

QTL analysis of grain β-glucan concentration

Single marker analysis of phenotypic data collected from the three year trials was done using the Qgene 3.0 software (Nelson 1997). With significance levels set at $p<0.1$ (three trials), $p<0.01$ (two trials) and $p<0.001$ (one trial), the analysis identified ten putative genomic regions for which multiple linked markers showed association with low β-glucan concentration (Table 5; Figure 2). For all putative QTLs, the CDC Bold alleles were favored and contributed to the phenotypic variances beyond 15%. The most significant QTL was *Qtl7.2*, which showed a significance level of $p<0.00001$ in all three trials. The *Qtl7.2* peak corresponded to markers mapped to the centromere region of chromosome 7H (Figure 3). In 2005, markers covering half of the 7H chromosome (35-115 cM) were positioned under *Qtl7.2* with peak LOD of 15.6 for the Bmag341 marker, which explained 55.7% of the variation in β-glucan concentration for that year. In the 2004 and 2006 trials, the LOD scores for *Qtl7.2* were lower (11.1, 44.3% explained and 5.9, 26.6% explained, respectively), but *Qtl7.2* was still the most significant QTL on the genome in these years. A less significant QTL (*Qtl7.1*) with a peak LOD score of 4.0 ($p<0.0001$) for marker pBb-4634 was indicated 41cM upstream of *Qtl7.2*, but association

between markers in the *Qtl7.1* region and trait data was only seen in the 2004 and 2005 trials (Figure 2).

Interval mapping using the MapQTL5 software (van Ooijen 2004) and phenotype data confirmed only *Qtl7.2* for all three trials (Figure 3). The three year LOD curves for *Qtl7.2* overlapped each other and peaked at approximately the same position (46-107 cM in 2004, 37-113cM in 2005 and 56-85 cM in 2006). The smaller *Qtl7.1* indicated upstream of Qtl7.2 by single marker analysis for 2004 and 2005 β-glucan values could not convincingly be distinguished from the *Qtl7.2* peak by interval mapping (Figure 3). Other QTLs identified on the genome by interval mapping were only seen in one or two of the trials. Low, but yet significant LOD scores of 3.20 (16.1% explained) and 3.83 (8.8% explained) were obtained for *Qtl3.2* on chromosome 3H for the years 2004 and 2005, respectively. Similarly, low significant LOD scores of 3.81 (25.4% explained) and 3.58 (17.5 % explained) were obtained for *Qtl.6.2* on chromosome 6H for two out of the three trials. The remaining QTLs suggested by single marker analysis (*Qtl2.1*, *Qtl2.2*, *Qtl3.1*, *Qtl4.1*, *Qtl5.1*, and *Qtl6.2*) did not reach threshold LOD scores determined for interval mapping or only showed LOD score above threshold value in one of the three field trials (*Qtl2.2*, *Qtl3.1*).

Development of targeted gene based markers:

To develop targeted gene based DNA markers we initiated a project to identify putative candidate genes that participate in beta-glucan synthesis. The reason for identifying candidate genes is because the identified markers are very tightly linked and can be directly used in barley improvement programs to develop barley lines with varying beta-glucan concentrations. We developed a comparative mapping based strategy to identify the putative candidate genes.

Design and mapping of primers from barley ESTs

The participation of rice Cellulose Synthase-Like F genes in (1,3;1,4)-beta-glucan biosynthesis has been recently shown (Burton et al. 2006). Rice genome sequences revealed that the *CslF* gene family contains eight genes. Six *CslF* genes on rice chromosome 7 were located on a syntenic region of barley 2H, which covered a major QTL for beta-glucan. The locations of the rest two *CslF* genes, *CslF6* and *CslF7*, were on rice chromosomes 8 and 10. However, the positions of these two genes in barley genetic map are currently not known. Although the *CslF* family was identified as the prime candidate of genes involved in beta-glucan synthase, *CslH* group was also considered to represent a prime candidate for beta-glucan synthesis (Farrokhi et al. 2006). A complete rice genome survey revealed the *CslF* family was most closely related to *CslD* group (Hazen et al. 2002), which includes five genes located on rice chromosomes 6, 8, 10, and 12.

In this study, a major QTL for beta-glucan concentration was mapped on the centromere region of barley chromosome 7H. Syntenic analysis between the barley consensus map and rice chromosomes indicated barley chromosome 7H and rice chromosomes 6 and 8 had common origin (Stein et al. 2007). In total 37 *Csl* families, 8 *Csl* genes distributed on these two rice chromosomes 6 and 8.

Using comparative analysis of barley ESTs and rice sequences, 49 primer pairs were designed for 18 cellulose synthase (*CesA*) and 31 *Csl* genes used to search candidate genes (Table 6). Two malt beta-glucan QTLs identified in a region between two RFLPs *Bhm1B* and *Amy2* in Steptoe × Morex population (Han et al. 2004). Five primer pairs were designed from these two genes (Han et al. 1997; Knox et al. 1987).

In order to identify positions on barley genetic map, polymorphism of 54 primer pairs between CDC Bold and TR251 were detected using 3% agarose gel, 6% polyacrylamide sequencing gel, and 10% polyacrylamide single-strand conformation polymorphism (SSCP) gel (Hongyo et al. 1993) (Fig.4). Only one primer, *CslA11.05* showed polymorphisms on sequencing gel and was mapped on chromosome 3H (Fig. 2). Three primers, *Amy2.03*, *Bhm1.01*, and *CslD5.01*, presented polymorphisms on SSCP gel and were assigned to 7H.

Discussion, Conclusions and Recommendations:

Barley beta-glucan is an important grain quality trait that influences grain utilization. Beta-glucan concentration is affected by both genotype and environment, therefore it is a quantitatively inherited trait. In addition, the structure of beta-glucan also varies between barley genotypes. In this project we focused on developing a genetic map and identifying chromosomal regions that affect beta-glucan concentration. We used QTL mapping to identify the chromosomal regions associated with beta-glucan concentration.

An important pre-requisite for most QTL mapping methods is the existence of at least one interval between two QTLs (Li et al., 2007). Therefore, it is imperative that high marker density maps are used to unambiguously identify genomic region associated with traits analyzed. Recently published genetic maps for crops largely rely on SSR markers, because of their high specificity and co-dominance, and these markers show fewer limitations imposed by previously used RFLP markers. The polymorphism between parent lines in the study was only 30% for SSR markers, which made it difficult to cover the whole barley genome with SSR markers alone. AFLP have limited applicability across genotypes, but are useful for increasing the genome coverage as these markers will generally generate a large number of polymorphic products per reaction (2 to 21 per primer pair in our study). Although the AFLP markers chosen for this study may have been biased towards AT-rich regions due to the specificity of the enzymes (*Eco*RI (G↓AATTC and *Mse*I (T↓TAA) used, no difference in the distribution of SSR and AFLP markers across the genome was observed. In contrast, mapped DArT markers tended to be more clustered (Figure 2), possibly explained by the DArT markers bias towards gene-rich regions (Wenzl et al., 2006)

Due to low polymorphism between parent lines, regions with few markers are often found on chromosome maps (Ramsay et al., 2000; Pillen et al., 2003; Li et al., 2006; Wenzl et al., 2006) and marker-scarce regions were also observed for maps developed in this study (Figure 2). For example, chromosome 2H was represented by two small linkage groups due to paucity of markers in the centromere region. Similarly, chromosome 4H spanned a 61 cM that corresponded to the centromere region and substantial segments from both telomeres of 4H were missing. In contrast to chromosome 4H, high marker density was observed for chromosome 7H, which may indicate that the parent lines are more divergent for this chromosome than the rest of the genome.

Marker distortion is commonly observed in mapping populations. In the CDC Bold × TR251 population we observed a relatively high marker distortion (42% at $p<0.05$), but comparable levels of distortions have been observed in other barley crosses. For example, marker distortions of 41% and 47% were found in barley populations studied by Marcel et al., (2007) and Stein et al., (2007), respectively. The unbalanced deviation from the expected allele frequency for certain loci could be due to gametophytic factors and/or sterility loci carried by one of the parents that resulted in low survival of certain recombinant lines.

The barley genetic map developed in this report consisted of 680 loci and measured 1,059 cM. The map compares well to four recent high-density consensus maps, 1,237 loci extending 1,211cM (Rostoks et al., 2005), 2,935 loci spanning 1,161 cM (Wenzl et al., 2006), 258 loci covering 1,081cM (Marcel et al., 2007), and 775 loci extending to 1,068 cM (Varshney et al., 2007). Only a few genetic maps in barley are in excess of 1,500 cM, including one of 1,873 cM with 282 loci (Becker et al., 1995), one of 1,900 cM with 171 loci (Buck-Sorlin, 2002), and another one of 1,596 cM with 1,172 loci (Hori et al., 2003). The significant differences in the barley genetic maps could be attributed to dissimilar genetic constitutions among different populations and/or mapping strategies, number of loci mapped, and mapping software used. For example, Gosselin et al., (2002) showed that when the same genotyping data was analyzed using Mapmaker or JoinMap, not only did the genetic length differ, but also the number of loci mapped onto each linkage group.

Single marker analysis revealed ten putative QTLs associated with grain β-glucan concentration (Table 5), but only Qtl7.2 at the centromere region on chromosome 7H was confirmed for all three field trials by interval mapping (Figure 3). Based on published chromosome (Varshney et al., 2007; Wenzl et al., 2006) and bin (http://barleygenomics.wsu.edu /nisInkage_maps/maps-svg.html) maps, the *Qtl7.2* peak identified in the CDC Bold × TR251 population is positioned to approximately the same region as two malt β-glucan QTLs identified in Steptoe × Morex population (Han et al., 1995, 2004) and QTL for grain β-glucan content in Beka × Logan population (Molina-Cano et al., 2007). Due to the broadness of *Qtl7.2* LOD curve and possible overlap with *Qtl7.1* located nearby, more than one locus on chromosome 7H may contribute to the low β-glucan phenotype favored by CDC Bold alleles. Although *Qtl7.2* was the dominant QTL for all thee trials, the magnitude of it's influence on β-glucan concentration varied for the three trials, which suggested an environmental impact on the locus/loci. The 7H QTL identified in the Beka × Logan population was also found to be largely dependent on the growth environment (Molina-Cano et al., 2007), which further suggests that this QTL is in common with *Qtl7.2* in our study. The location of *Qtl7.2* peak corresponds to markers Bmac31, Bamg341, bpb-5456, bpb-1476, and bpb-0125, for which no recombinants were identified among the 88 DH lines genotyped. Further fine mapping of the *Qtl7.2* region using the whole CDC Bold × TR251 mapping population will be needed to delineate *Qtl7.2* to a smaller segment in order to resolve the region into one or multiple QTLs.

The putative QTLs indicated for chromosomes 2H, 3H 4H, 5H and 6H (Table 5) were unstable in the three year trials, which may indicate strong interactions among trait alleles underlying these putative QTLs and environmental factors. Some of these putative QTLs corresponded to regions associated with malt or grain β-glucan concentration in previous studies. Based on common markers, the *Qtl2.1* shares a similar chromosomal location as a cellulose-synthase like gene (*CslF*) on chromosome 2H. The *CslF* gene product has been shown to catalyze β-glucan synthesis when expressed in *Arabidopsis*, a plant that normally does not produce β-glucan (Burton et al., 2006). Common markers at the *Qtl2.2* locus suggest that it may correspond to QTL locus malt β-glucan in un-germinated grain of Steptoe × Morex population (Han et al., 1995). *Qtl3.2* on chromosome 3H and *Qtl6.1* on chromosome 6H correspond in location to QTL loci for extract β-glucan identified in Harrington × TR306 population (Igartua et al., 2000). A QTL affecting malt β-glucan concentration was reported for the telomere region on the short arm of chromosome 4H in the Steptoe × Morex population (Han et al., 1995; Gao et al., 2004). However, any QTL for β-glucan concentration in this region may have eluded detection due to lack of polymorphism for this segment of the 4H chromosome.

All ten putative QTLs indicated to be associated with barley grain β-glucan were derived from the low β-glucan parent, CDC Bold. Since CDC Bold carries β-glucan alleles desired by the malting and feed industries, genetic markers associated with the identified QTLs could be used for marker assisted breeding of lines with low β-glucan concentration. Due to the environmental impact on β-glucan concentration, QTLs showing the highest stability across different environments would be preferred for barley improvement.

Conclusions:

A major conclusion of this research is the identification of *Qtl7.2* associated with beta-glucan concentration. We used comparative genomics (rice, Arabidopsis and barley) to identify putative candidate genes that map to *Qtl7.2*. Cellulose synthase genes were the most likely targets based on the similarities between beta-glucan and cellulose biosynthesis. We designed more than fifty oligonucleotide primers to try to identify cellulose synthase like genes that map to *Qtl7.2*. We obtained some encouraging results, with three genes mapping to the identified QTL. However, we could not complete this research due to lack of time.

- Developed a barley (CDC Bold) genetic map with 680 loci and 1,059 cM long.
- Identified ten putative QTL associated with barley beta-glucan concentration. A *Qtl7.2* was confirmed to be stable over all the three field trials.
- Located several potential candidate genes to the *Qtl7.2*.
- Showed by mapping that a cellulose synthase like gene could be mapped to the chromosome 7 where *Qtl7.2* is located.
- Tried to validate selected SSR markers in *Qtl7.2* but met with limited success

Future research/Recommendations: The results obtained in this project have laid the foundation for identifying putative candidate genes (cellulose synthase gene family) that participate in bet-glucan biosynthesis. Detailed expression analyses will identify the cellulose synthase or a similar glucan synthase gene responsible for beta-glucan synthesis. This will enable us to understand the biosynthesis of non-starch complex carbohydrates using beta-glucan as a model. The results from barley can then be applied to other cereals such as oat and wheat.

A second approach to utilize the genetic map is to saturate the QTL regions with more markers to precisely identify the chromosomal regions associated with beta-glucan synthesis.It is anticipated that fine mapping will result in markers more closely related to beta-glucan concentration. However, the genetic map developed in this project can be used as a resource for genetic analyses of other traits of interest to barley improvement programs.

Acknowledgements: The funding and support from the Saskatchewan Agriculture and Food was acknowledged in all the research publications and presentations made at conferences and seminars. We also thank Dr. W.L. Legge, AAFC Brandon Research Centre for providing the doubled haploid mapping population used in the genetic mapping studies. We thank Dr. Marta Izydorczyk, Grain Research Laboratory, Canadian Grain Commission, Winnipeg for analysis of beta-glucan structure and its variation in barley genotypes.

Literature Cited:

Aastrup, S., Jorgensen, K.G., 1988. Application of the calcoflour flow injection analysis method for determination of β-glucan in barley, malt wort and beer. Journal of the American Society of Brewing Chemists 46, 76-81.

Ayoub, M., Mather, D.E., 2002. Effectiveness of selective genotyping for detection of quantitative trait loci an analysis of grain and malt quality traits in three barley populations. Genome 45, 116-24.

Båga, M., Chodaparambil, S.V., Limin, A.E., Pecar, M., Fowler, D.B., Chibbar, R.N., 2007. Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. Functional & Integrative Genomics 7, 53-68.

Backes, G., Orabil, J., Fischbeck, G., Jahoor, A., 2006. Barley. In Kole (Ed.) Genome mapping and molecular breeding in plants Volume 1, Cereals and Millets, Springer Press, pp.155-209.

Becker, J., Vos, P., Kuiper, M., Salamini, F., Heun, M., 1995. Combined mapping of AFLP and RFLP markers in barley. Theoretical and Applied Genetics 249, 65-73.

Buck-Sorlin, G.H., 2002. The search for QTL in barley (*Hordeum vulgare* L.) using a new mapping population. Cellular and Molecular Biology Letters 7(2A), 523-35.

Buckeridge, M.S., Vergara, C.E., Carpita, N.C., 2001. Insight into multi-site mechanisms of glycosyl transfer in (1→4)-β-D-glycans provided by the cereal mixed-linkage (1→3),(1→4)-β-D-glucan synthase. Phytochemistry 57, 1045-1053.

Burger, W.C., LaBerge, D.E., 1985 Malting and brewing quality. In Rasmusson, D. C. (ed), Barley. American Society of Agronomy, Madison, WI, pp. 367-401.

Burton, R.A., Farrokhi, N., Bacic, A., Fincher, G.B., 2005 Plant cell wall polysaccharide biosynthesis: real progress in the identification of participating genes. Planta 221, 309-312.

Burton, R.A., Shirley, N.J., King, B.J., Harvey, A.J., Fincher, G.B., 2004 The *CesA* gene family of barley. Quantitative analysis of transcripts reveals two groups of co-expressed genes. Plant Physiology 134, 224-236.

Burton, R.A., Wilson, S.M., Hrmova, M., Harvey, A.J., Shirley, N.J., Medhurst, A., Stone, B.A., Newbigin, E.J., Bacic, A., Fincher, G.B., 2006. Cellulose synthase-like *CslF* genes mediate the synthesis of cell wall (1,3;1,4)-β-D-glucans. Science 311, 1940-1942.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M., 2002. High (1→3),(1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. Journal of Cereal Science 36, 59-56.

Churchill, G.A., Doerge, R.W., 1994. Empirical threshold values for quantitative trait mapping. Genetics 138, 963-971.

Fincher, G.B., 1975. Morphology and chemical composition of barley endosperm cell walls. Journal of the Institute of Brewing 81, 116-122.

Fox, G.P., Panozzo, J.F., Li, C.D., Lance, R.C.M., Inkerman, P.A., Henry, R.J., 2003. Molecular basis of barley quality. Australian Journal of Agricultural Research 54, 1081-1101.

Gao, W., Clancy, J.A., Han, F., Jones, B.L., Budde, A., Wesenberg, D.M., Kleinhofs, A., Ullrich, S.E., 2004. Fine mapping of a malting-quality QTL complex near the chromosome 4H S telomere in barley. Theoretical and Applied Genetics 109, 750-760.

Han, F., Clancy, J.A., Jones, B.L., Wesenberg, D.M., Kleinkofs, A., Ullrich, S.E., 2004.

Dissection of a malting quality QTL region on chromosome 1 (7H) of barley. Molecular Breeding 14, 339-347.

Han, F., Ullrich, S.E., Chirat, S., Menteur, S., Jestin, L., Sarrafi, A., Hayes, P.M., Jones, B.L., Blake, T.K., Wesenberg, D.M., Kleinhofs, A., Kilian, A., 1995. Mapping of β-glucan content and α-glucanase activity loci in barley-grain and malt. Theoretical and Applied Genetics 91, 921-927.

Han, F., Ullrich, S.E., Kleinhofs, A., Jones, B.L., Hayes, P.M., Wesenberg, D.M., 1997 Fine structure mapping of the barley chromosome-1 centromere region containing malting-quality QTLs. Theoretical and Applied Genetics 95, 903-910.

Hayes, P.M., Castro, A., Marquez-Cedillo, L., Corey, A., Henson, C., Jones, B.L., Kling, J., Mather, D., Matus, I., Rossi, C., Sato, K., 2003 Genetic diversity for quantitative inherited agronomic and malting quality traits. In van-Bothmer, R., van-Hintum, T., Knüpffer, H. and Sato, K. (eds), Diversity in barley. Elsevier Science BV, Amsterdam, the Netherlands, pp. 201-226.

Hayes, P.M., Liu, B.H., Knapp, S.J., Chen, F., Jones, B., Blake, T., Franckowiak, J., Rasmusson, J., Sorrells, M., Ullrich, S.E., Wesenberg, D., Kleinhofs, A., 1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theoretical and Applied Genetics 87, 392-401.

Hecker, K.D., Meier, M.L., Newman, R.K., Newman, C.W., 1998. Barley β-glucan is effective as a hypocholesterolemic ingredient in foods. Journal of the Science of Food and Agriculture 77, 179-183.

Holland, N., Holland, D., Helentjaris, T., Dhugga, K.S., Xoconostle-Cazares, B., Delmer, D.P., 2000 A comparative analysis of the plant cellulose synthase (*CesA*) gene family. Plant Physiology 123, 1313-1323.

Hongyo, T., Buzard, S.G., Calvert R.J., Weghorst, m.C., 1993. 'Cold SSCP': a simple, rapid and non-radioactive method for optimized sinle-strand conformation polymorphism analyses. Nucleic Acids Research 21, 3637-3642.

Hori, K., Kobayashi, T., Shimizu, A., Sato, L., Takeda, K., Kawasaki, S., 2003. Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theoretical and Applied Genetics 107, 806-813.

Igartua, E., Edney, M., Rossnagel, B.G., Spaner, D., Legge, W.G., Scoles, G.J., Eckstein, PE., Penner, G.A., Tinker, N.A., Briggs, K.G., Falk, D.E., Mather, D.E., 2000. Marker-based selection of QTL affecting grain and malt quality in two-row barley. Crop Science 40, 1426-1433.

Izydorczyk, M.S., Storsley, J., Labossiere, D., MacGregor, A.W., Rossnagel, B.G., 2000. Variation in total and soluble beta-glucan content in hulless barley: effects of thermal, physical, and enzymic treatments. Journal of Agriculture and Food Chemistry 48, 982-989.

Jaccoud, D., Peng, K., Feinstein, D., Kilian, A., 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29, e25

Kosambi, D.D., 1943. The estimation of map units from recombination values. Annals of Eugenics 12, 172-175.

Li, Huihui., Ye, Guoyou., Wang, Jiankang., 2007. A modified algorithm for the improvement of composite interval mapping. Genetics 175, 361-374.

Li, J.Z., Huang, X.Q., Heinrichs, F., Ganal, M.W., Röder, M.S., 2006. Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross

population of spring barley. Genome 49, 454-466.

Liu, Z.W., Biyashev, R.M., Maroof, M.A.S., 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics 93, 869-876.

Marcel, T.C., Varshney, R.K., Barbieri, M., Jafary, H., de, Kock, M.J.D., Graner, A., Niks, R.K., 2007. A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theoretical and Applied Genetics 114, 487-500.

Mather, D.E., Tinker, N.A., LaBerge, D.E., Edney, M., Jones, B.L., Rossnagel, B.G., Legge, W.G., Briggs, K.G., Irvine, R.B., Falk, D.E., Kasha, K.J., 1997. Regions of the genome that affect grain and malt quality in a North American two-row barley cross. Crop Science 37, 544-554.

Molina-Cano, J.L., Moralejo, M., Elia, M., Munoz, P., Russell, J.R., Perez-Vendrell, A.M., Ciudad, F., Swanston, J.S., 2007. QTL analysis of a cross between European and North American malting barleys reveals a putative candidate gene for β-glucan content on chromosome 1H. Molecular Breeding 19, 275-284.

Nelson, J., 1997. Qgene: software for marker-based genomic analysis and breeding. Molecular Breeding 3, 239-245.

Pear, J.R., Kawagoe, Y., Schreckengost, W.E., Delmer, D.P., Stalker, D.M., 1996 Higher plants contain homologs of the bacterial *celA* genes encoding the catalytic subunit of cellulose synthase. Proceedings of the National Academy of Sciences of the United States of America 93, 12637-12642.

Pillen, K., Zacharias, A., J., Léon., 2003. Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics 107, 340-352.

Poppitt, S.D., van, Drunen, J.D., McGill, A.T., Mulvey, T.B., Leahy, F.E., 2007. Supplementation of a high-carbohydrate breakfast with barley β-glucan improves postprandial glycaemic response for meals but not beverages. Asia Pacific Journal of Clinical Nutrition 16, 16-24.

Ramsay, L., Macaulay, M., Ivanissevich, degli, S., MacLean, K., Cardle, L., Fuller, J., Edwards, K.J., Tuvesson, S., Morgante, M., Massari, A., Maestri, E., Marmiroli, N., Sjakste, T., Ganal, M., Powell, W., Waugh, R., 2000. A simple sequence repeat-based linkage map of barley. Genetics 156, 1997-2005.

Rostoks, N., Mudie, S., Cardle, L., Russell, J., Ramsay, L., Booth, A., Svensson, J.T., Wanamaker, S.I., Walia, H., Rodriguez, E.M., Hedley, P.E., Liu, H., Morris, J., Close, T.J., Marshall, D.F., Waugh, R., 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Molecular Genetics and Genomics 274, 515-527.

Seefeldt, F.H., van den Berg, F., Kockenberger, W., Engelsen, S.B., Wollenweber, B., 2007. Water mobility in the endosperm of high β-glucan barley mutants as studied by nuclear magnetic resonance imaging. Journal of Magnetic Resonance Imaging 25, 425-432.

Stein, N., Prasad, M., Scholz, U., Thiel, T., Zhang, H., Wolf, M., Kota, R., Varshney, R.K., Perovic, D., Grosse, I., Graner, A., 2007. A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theoretical and Applied Genetics 114, 823-839.

Stewart, D.C., Hawthorne, D., Evans, D.E., 1998. Cold sterile filtration: A small-scale filtration and investigation of membrane plugging. Journal of the Institute of Brewing

104, 321-326.

Switala, K.J., Schick, K.G., Griffith, A., Sebree, B.R., 1989. Rapid determination of β-glucan in wort by flow injection analysis. Journal of the American Society of Brewing Chemists 47, 54-56.

Tanaka, K., Murata, K., Yamazaki, M., Onosato, K., Miyao, A., Hirochika, H., 2003 Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. Plant Physiology 133, 73-83.

van Ooijen, J.W., 2004. MapQTL 5: software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, The Netherlands.

van Ooijen, J.W., R.E., Voorrips., 2001. JoinMap® 3.0: Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.

Varshney, R.K., Marcel, T.C., Ramsay, L., Russell, J., Röder, M.S., Stein, N., Waugh, R., Langridge, P., Niks, R.E., Graner, A., 2007. A high density barley microsatellite consensus map with 775 SSR loci. Theoretical and Applied Genetics 114, 1091-1103.

vos P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M, Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407-14.

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., Kilian, A., 2004. Diversity arrays technology (DArT) for whole-genome prowling of barley. Proceedings of the National Academy of Sciences USA 101, 9915-9920.

Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K.P., Muehlbauer, G.J., Chalmers, K.J., Kleinhofs, A., Huttner, E., Kilian, A., 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and phenotypic traits. BioMed Central Genomics 7, 206-228.

Youngs, H., Hamann, T., Osborne, E., Somerville, C., 2007 The cellulose synthase superfamily. In Brown RM Jr. and Saxena IM. (eds.), Cellulose: molecular and structural biology. Springer Press pp.35-48.

Zale, J.M., Clancy, J.A., Ullrich, S.E., Jones, B.L., Hayes, P.M., 2000. Summary of barley malting-quality QTLs mapped in various populations. Barley Genetics Newsletter 30, 44-54.

(h) **Appendices** – nil

(i) **Other** – (e.g. papers produced; conference presentations made; personnel involved)

Papers Produced:

Li, J., Bäga, M., Rossnagel, B.G., Legge, W.G., and Chibbar, R.N. 2008 Identification of quantitative trait locus for β-Glucan Concentration in barley grain J of Cereal Science (submitted).

Ganeshan, S., Dahleen, L.S., Tranberg, J., Lemaux, P.G. and Chibbar, R.N. 2008. Barley In: Kole C, Hall TC (eds) A Compendium of Transgenic Crop Plants. Wiley-Blackwell (in press)

Conference Presentations:

Ravindra N Chibbar, M. Bäga, S. Ganeshan 2007. Genomic strategies to modify grain

storage carbohydrates to diversify crop utilization. International seminar on Food Security, Biomass Energy and Livelihood Strategies, African Asian Studies Promotion Association, Georg-August-University, Goettingen, Germany, November 18-20, 2007

Ravindra Chibbar 2007. Genomic strategies to modify grain storage carbohydrates to diversify utilization of cereal and pulse crops. Department of Biochemistry, Memorial University of New Foundland, St. Johns New Foundland, Canada. October 26, 2007

Båga M, Li J, Chung C, Ansari O, Legge WG, Rossnagel BG, Chibbar RN. Genetic characterization of starch granule size and β-glucan concentration in barley grain. BMBRI's 20th Triennial Barley Improvement Meeting, Leduc, Alberta, July 31 – Aug 2, 2007.

Ravindra N Chibbar, Monica Båga, Seedhabadee Ganeshan, Pierre Hucl, Jingzhao Li, Rajender Singh, Eric K Asare, Jenna Drinkwater, Brij Verma and Brian G Rossnagel 2007. Genomic strategies to modify storage carbohydrates to diversify utilization of cereal grains. International Conference on Sustainable Agriculture for food, bioenergy and livelihood security. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) India. February 14-16, 2007.

Li, J, M. Båga, B.G. Rossnagel, W.G. Legge, R.N. Chibbar 2007. Identification of quantitative trait loci for β-glucan concentration in barley. Soils and Crops Workshop, University of Saskatchewan, March, 1-2, 2007 Saskatoon, Sask.

Li, J, M. Båga, B.G. Rossnagel, W.G. Legge, R.N. Chibbar 2007. Identification of quantitative trait loci for β-glucan concentration in barley. Plant and Animal Genome XV San Diego, California, USA. Jan 13-17, 2007.

Ravindra N Chibbar 2006. Grain carbohydrate modification for human health, food and animal feed application. 4th Food Science International Symposium Xiamen, Fujian, China October 23-26, 2006.

Ravindra N Chibbar, Monica Båga, Seedhabadee Ganeshan, Jingzhao Li, Rajender Singh, Eric K Asare, Jenna Drinkwater, Brian G Rossnagel and Pierre Hucl 2006. Genomic strategies to improve starch and non-starch carbohydrates in cereal grains. The 1st International Forum on Cereal Science "Cereal Foods: Cooperation and Opportunities across Borders" Southern Yangtze University, Wuxi, China, October 18-21, 2006.

Ravindra N Chibbar 2006. Molecular biological strategies to alter complex carbohydrates in wheat and barley. Plant Polysaccharide Workshop 2006 – Carbohydrates for food and fuel. Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada, September 21-23, 2006.

Personnel:

Dr. Monica Båga	Research Scientist
Dr. Jingzhao Li	Post-doctoral Fellow
Marin Pecar	Technician
Shuhua Zou	Technician

Legends for Figures

Figure 2. Genetic linkage map for barley cross CDC Bold × TR251.

The genetic distances in centiMorgans (*cM*) Kosambi mapping units are shown on the *left* side of the chromosome bar. Approximate location of centromere (C) region is indicated. Locus names are indicated on the *right* side of the chromosome bar. Loci showing association with low *β*-glucan concentration in grain are followed by asterisks indicating level of significance as follows: ***: $p<0.01$, ****: $p<0.005$, *****: $p<0.001$, ******: $p<0.0005$, *******: $p<0.0001$.

Figure 3. Test-statistic scans from interval mapping of *β*-glucan concentration in grain.

QTL mapping data for 170 DH lines from the CDC Bold × TR251 population tested in three years. Barley chromosomes ordered from 1H to 7H are represented by *solid vertical lines* (*y*-axis) with genetic distances (cM) indicated. LOD scores (*x*-axis) are displayed by *solid curves* along the chromosomes. The LOD threshold for each field trial is represented by a *dashed vertical line* with genome-wide significance threshold indicated ($p=0.05$). The threshold values were estimated by 1,000-permutations.

Table 5. Putative QTLs for low β-glucan concentration as indicated by single marker analysis.

QTL	Chromosome	Region (cM)	Peak marker[a]	Source[b]	Significance[c]			LOD[d]	Mean[d,e]		Phenotypic Variance (%)[d,f]
					2004	2005	2006		CDC Bold	TR251	
Qtl2.1	2H	0 – 3	E32M62.1	CDC Bold	***	*******	****	4.4	3.4	3.8	20.9
Qtl2.2	2H	24 – 35	bPb-6963	CDC Bold	**	*******	****	3.7	3.5	3.9	18.4
Qtl3.1	3H	17 – 32	bPb-3565	CDC Bold	*******	****	***	4.5	3.9	4.4	21.4
Qtl3.2	3H	68 – 74	E33M47.9	CDC Bold	******	*******	***	3.6	3.4	3.8	17.9
Qtl4.1	4H	11	bPb-6096	CDC Bold	******	****	****	3.3	3.8	4.3	16.9
Qtl5.1	5H	127	E37M62.3	CDC Bold	***	******	**	3.1	3.6	3.9	15.0
Qtl6.1	6H	16 – 32	E38M61.4	CDC Bold	**	*******	*******	3.4	4.0	4.4	16.2
Qtl6.2	6H	110 – 122	bPb-2410	CDC Bold	*****	*******	***	3.3	3.5	3.9	16.3
Qtl7.1	7H	21 – 29	bPb-4634	CDC Bold	****	*******	ns	4.1	3.6	4.1	19.6
Qtl7.2	7H	35 – 115	Bmag341	CDC Bold	*******	*******	*******	15.6	3.4	4.1	55.7

[a]Marker with highest p-value

[b]Parent line contributing to low β-glucan concentration

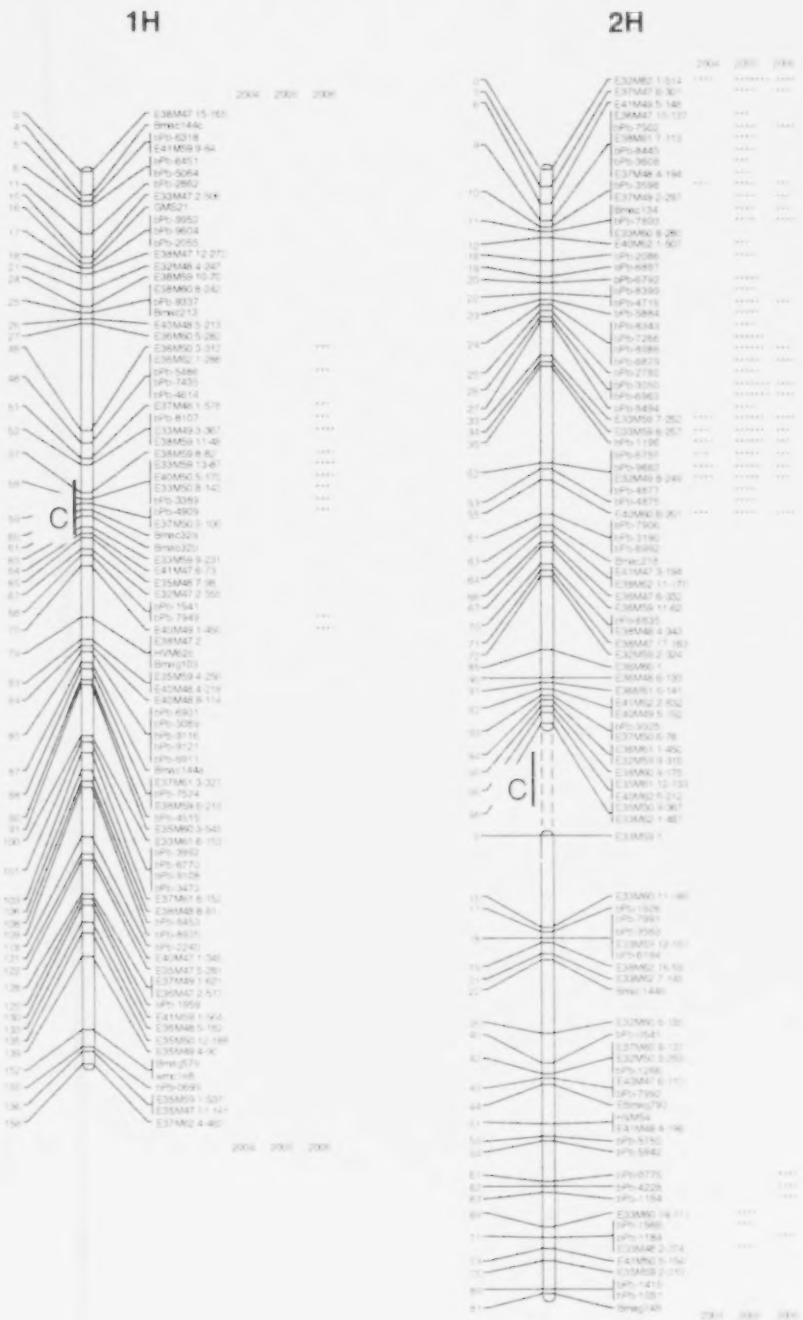
[c]p-value with following significance levels: *: $p<0.1$, **: $p<0.05$, ***: $p<0.01$, ****: $p<0.005$, *****: $p<0.001$, ******: $p<0.0005$, *******: $p<0.0001$, ns: not significant

[d]LOD score, mean, and phenotypic variance from year with underlined significance value.

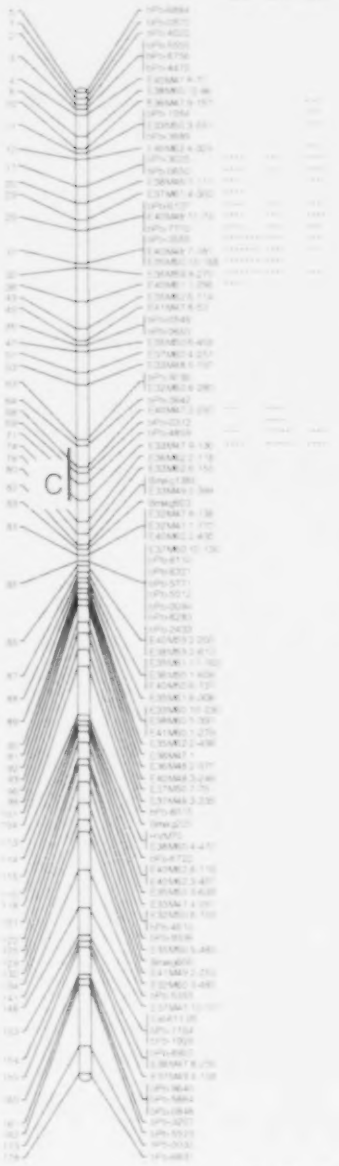
[e]Phenotypic mean value for β-glucan concentration.

[f]The percent phenotypic variance associated with each significant QTL was calculated from the regressions of each maker/phenotype combination based on data from year with the underlined significance value.

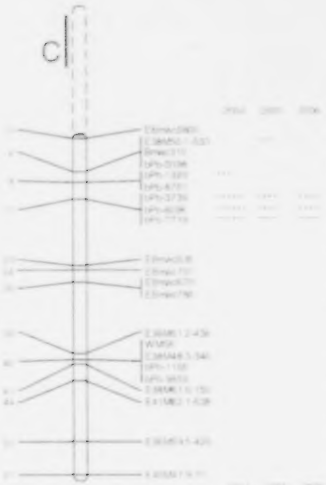
Figure 2. Genetic linkage map for barley cross CDC Bold × TR251.



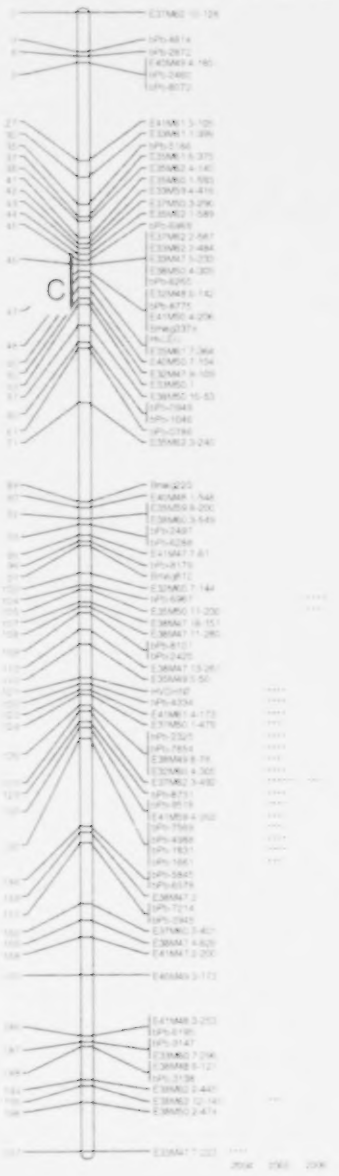
3H

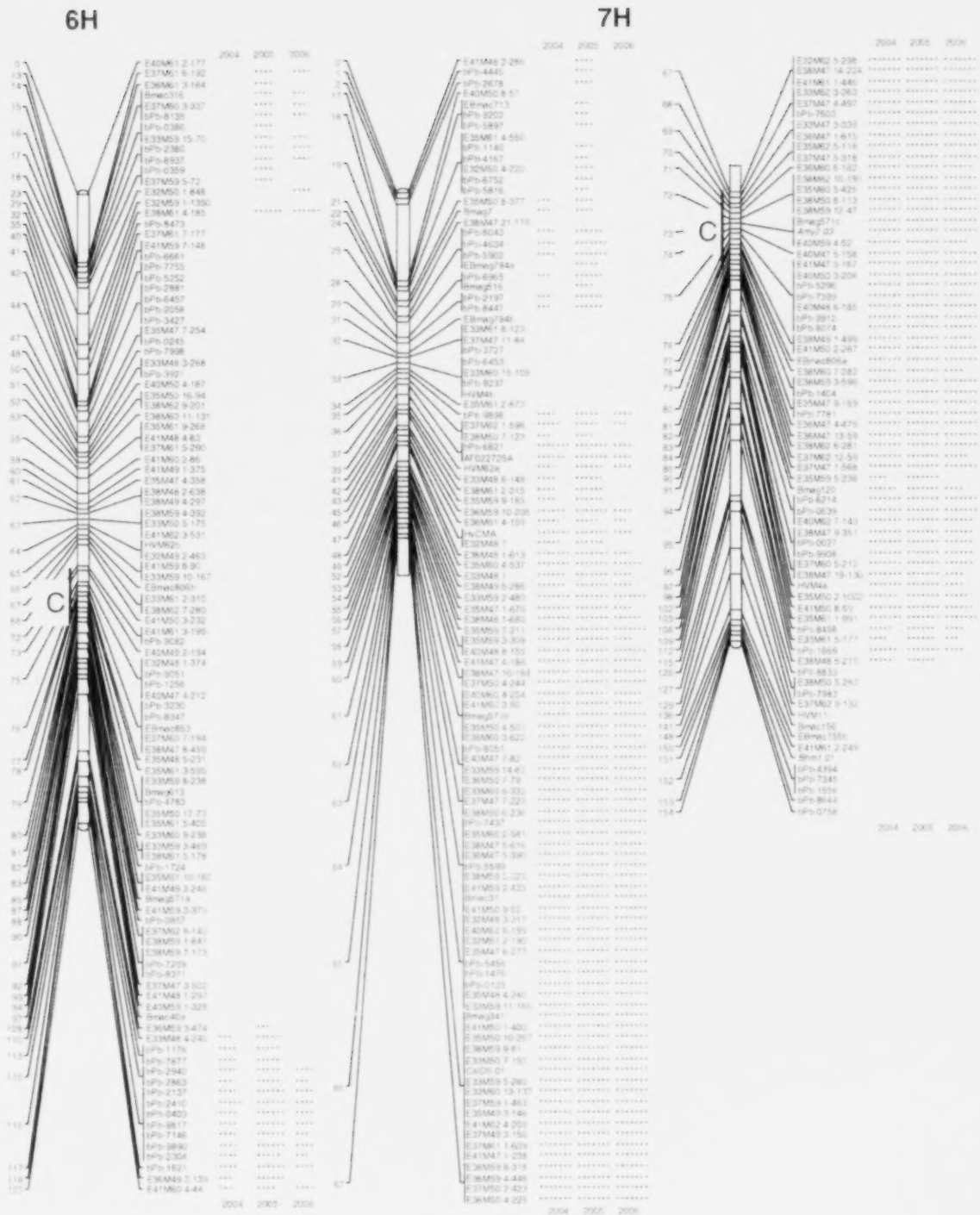


4H



5H




6H
7H
C
C

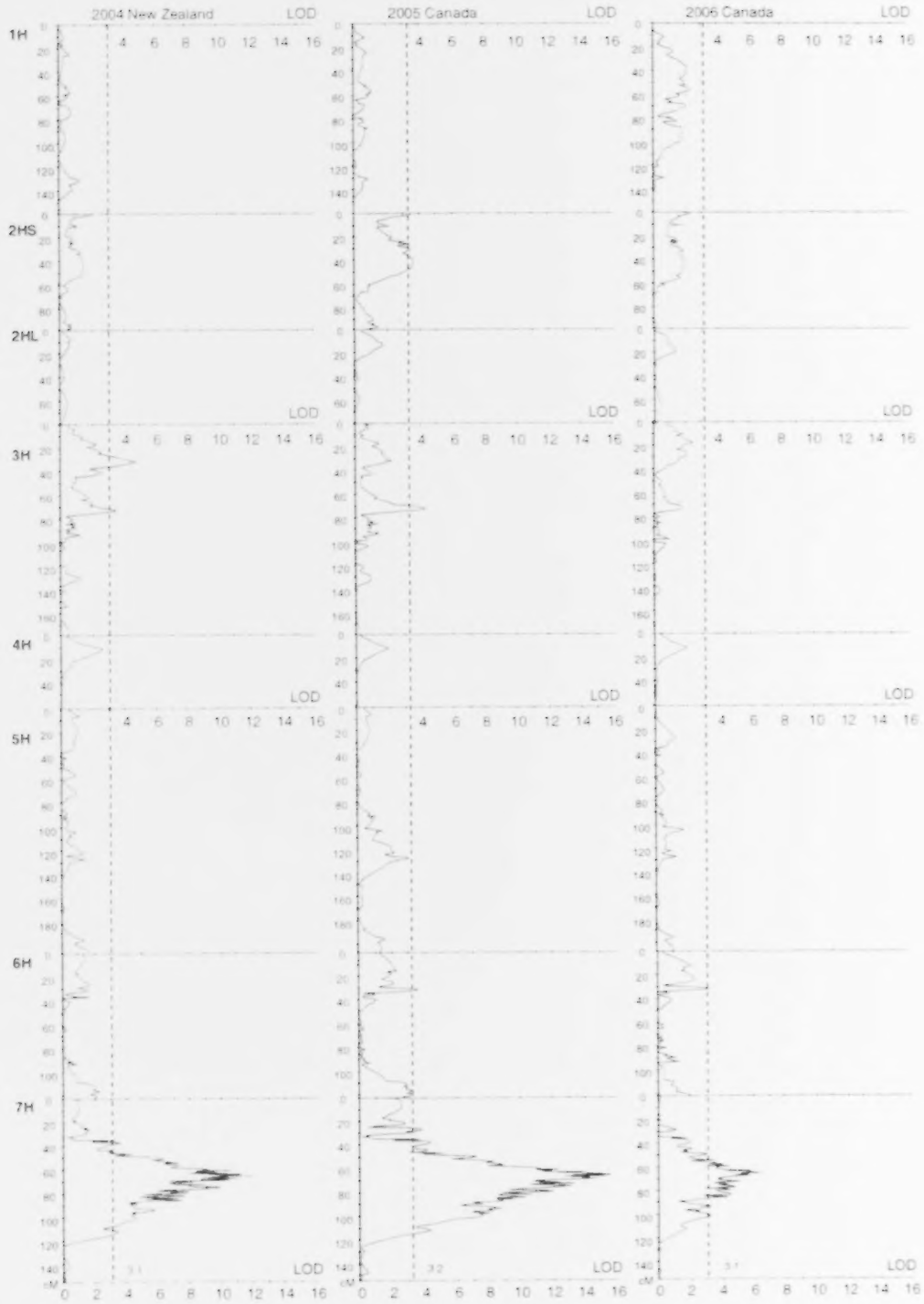


Figure 3. Test-statistic scans from interval mapping of β-glucan concentration in grain.

Table 6. EST-based Primer designed

	Primer	Expected size	Sequence	Name	T_m
1	*CSLC9.01*	382 bp	AGGTGGAGCAGCAGGTGAATGGTG	*CslC9.01F*	63°C
			GATGTCCGGTATGCAAAGCCTGAA	*CslC9.01R*	
2	*CSLC9.02*	393 bp	TCCTCCCGATGACGATGTTTG	*CslC9.02F*	57°C
			GCGCCAGCTCCTTCTTGTAT	*CslC9.02R*	
3	*CSLC7.01*	324 bp	ACAAGGCTGGAAATCTCAAGTCTG	*CslC7F*	55°C
			AGCCTGGTTAACAAGTTCTCATCC	*CslC7R*	
4	*CSLD5.01*	198 bp	GTCGGCGCCTTCCTCGTCCAGTTC	*CslD5.01F*	65°C
			TCTCCATCACCACGCCCTCAAAAG	*CslD5.01R*	
5	*CSLD2.01*	423 bp	GGCCCGCTACCTGGATTCAAC	*CslD2F*	60°C
			CACCGTCACCACCATACAGAGGAT	*CslD2R*	
6	*CSLA9.01*	218 bp	CCAAATCCCCATGTACAACGAGAG	*CslA9.01F*	60°C
			CCTATCCGACGGCCACGACAG	*CslA9.01R*	
7	*CSLA9.02*	475 bp	CCATATCTCGCACGTAATCCACAA	*CslA9.02F*	58°C
			ATGAACCTGCCTCCTGCTCTACTT	*CslA 9.02R*	
8	*CSLC10.01*	572 bp	CGACGCCGGCTACTACCC	*CslC10.01F*	55°C
			CTTGCACCAGCCCCAGTTCAT	*CslC10.01R*	
9	*CSLC10.02*	180 bp	CGACGCCGGCTACTACCC	*CslC10.02F*	55°C
			GGCCGCGATTGATTGCTGATAC	*CslC10.02R*	
10	*CSLC10.03*	306 bp	TCGCGAAGTGGCAGCAGACC	*CslC10.03F*	60°C
			GCACCAGCCCCAGTTCATCGTT	*CslC10.03R*	
11	*CSLC10.04*	394 bp	GTGGAGCAGCAGGTGAATGGTGTG	*CslC10.04F*	62°C
			ATGTCCGGTATGCAAAGCCTGAAT	*CslC10.05R*	
12	*CSLC10.05*	600 bp	CCCGAGTCATACGAGGCTTACAGG	*CslC10.05F*	60°C
			GCTTCGGGCACAAACATCGTCA	*CslC10.05R*	
13	*CSLC10.06*	220 bp	GAGCGCCTACGAGTGGGTGGTG	*CslC10.06F*	63°C
			GGCGGCCGTCAGCAGGAG	*CslC10.06R*	
14	*CSLF4.01*	286 bp	CATCCTCCTGCATCCCTACA	*CslF4.01F*	52°C
			TCCCCGACGACTGACATTG	*CslF4.01R*	
15	*CSLF9*	1737 bp	GGCCAAGATGCGCGGGGAGTA	*CslF9.01F*	65°C
			GCGGAAGGCCTGCGAGTTGTTG	*CslF9.01R*	
16	*CSLD3.01*	810 bp	CTATGTCGGCACTGGCTGTCTGTT	*CslD3.01F*	60°C
			CCGCGGAAGGCATCACG	*CslD3.01R*	
17	*CSLD3.02*	272 bp	TCTGCGCCGGACTTGGATGTG	*CslD3.02R*	63°C
			CATGCTGGCGGTGCTGGAGA	*CslD3.02F*	
18	*CSLA11.01*	353 bp	GGCGGCGGTGGCGGAGGA	*CslA11.01F*	65°C
			TCTGGACGAGCACGACGGGGAA	*CslA11.01R*	
19	*CSLA11.04*	925 bp	AACTTTCAACTTTTCCATCAC	*CslA11.04F*	55°C
			CGTACCCGTGCTTCATCC	*CslA11.04R*	
20	*CSLA11.05*	341 bp	CCGTTGTCCGTTTTCTTCCCTGAG	*CslA11.05F*	62°C
			CATGGCTGGCTTCGTCTTGTTG	*CslA11.05R*	
21	*CSLA11.06*	672 bp	CCGTTGTCCGTTTTCTTCCCTGAG	*CslA11.06F*	62°C
			TAGTATCCCTTGTTTGCGTAGAGC	*CslA11.06R*	
22	*CSLF6.01*	306 bp	TCTACGCCAACCACAACCGCATCT	*CslF6.01F*	63°C
			CCGTACGTCTTCTTGGGCAGTGG	*CslF6.01R*	
23	*CSLF6.02*	468 bp	GCTCAACCAACTCCCAAAGT	*CslF6.02F*	60°C
			TGCTTGATGTCATGCTCCAT	*CslF6.02R*	
24	*CSLF6.03*	703 bp	ATGGAGCATGACATCAAGCA	*CslF6.03F*	60°C
			GCAGCACCTGCACATTTTTA	*CslF6.03R*	
25	*CSLF6.04*	414 bp	AGCACGGAAAGACGCCAGTC	*CSLF6.04F*	60°C
			TCCCTTGTTGTTCGCGGAAT	*CSLF6.04R*	
26	*CSLE1.01*	270 bp	TCGCCGGCCGCTTACTTCT	*CslE1.01F*	60°C
			CGTGTCTATTCGCTCGGTCAT	*CslE1.01R*	

27	*CSLE1.02*	535 bp	TCGCCGGCCGCTTACTTCT	*CslE1.02F*	60°C
			GCGCATTTCCTTCATTATCAACTG	*CslE1.02R*	
28	*CSLE1.03*	588 bp	AAGAGGCCTCAGCACCACCATAA	*CslE1.03F*	59°C
			GACCGCATCTTTGTTGTTGGAGTA	*CslE1.03R*	
29	*CSLE1.04*	266 bp	GATGCGCTGTGCTTCTTCC	*CslE1.04F*	53°C
			GGCCGCCCAAACTGTCC	*CslE1.04R*	
30	*CSLE1.05*	659 bp	CCCTTCACTGGCCCTCCTCA	*CslE1.05F*	59°C
			GACGACCCGAATCCCATAATCTCT	*CslE1.05R*	
31	*CSLD4.01*	173 bp	ATCGCCGGCGTGGACATCTC	*CslD4.01F*	62°C
			GCCACCGCGTTCAGCATCATAAT	*CslD4.01R*	
32	*CESA8.01*	432 bp	GTCTGCCGCCCCTGCTACG	*CESA8.01F*	60°C
			CCATCATCGGCATCAGTGTCGTC	*CESA8.01R*	
33	*CESA8.02*	377 bp	GATACGTCCCTTCAGTCAC	*CESA8.02F*	55°C
			CTTCATTTTCCAGCCATCAACTCT	*CESA8.02R*	
34	*CESA8.03*	251 bp	CATATGATGTCCCCTACTGG	*CESA8.03F*	55°C
			GAATTGCACCCTTGTCCTG	*CESA8.03R*	
35	*CESA8.04*	686 bp	TGGAAAATGAAGCAGGACA	*CESA8.04F*	55°C
			AGAACAACCAACCGCAGAACAATG	*CESA8.04R*	
36	*CESA8.05*	265 bp	CCAATCAACCGGGAGACCTACC	*CESA8.05F*	58°C
			CACGGGATAATCAACAGCAAGAAT	*CESA8.05R*	
37	*CESA8.06*	266 bp	AGAAGTATGACATCGAACCCAGAG	*CESA8.06F*	53°C
			CCTCGGGGACTTTCAATG	*CESA8.06R*	
38	*CESA8.07*	337 bp	GGCCAGGAAACAATACCAG	*CESA8.07F*	55°C
			AATCCAGGACGCTTTTCACG	*CESA8.07R*	
39	*CESA8.08*	333 bp	CCCCGTTTGGTCTATGTGTCTCGT	*CESA8.08F*	60°C
			CATCAAACCTCTGTGGGAACTGC	*CESA8.08R*	
40	*CESA8.09*	413 bp	CGTGTCTCGGCTGTCCTT	*CESA8.09F*	55°C
			ATAAACTGGCCCTTGAATG	*CESA8.09R*	
41	*CESA8.10*	313 bp	GTGTGGGGGCAAGAAGAA	*CESA8.10F*	55°C
			CATCAGAGTGGAGGCAACAA	*CESA8.10R*	
42	*CESA8.11*	319 bp	CATTTGTTGCCTCCACTCT	*CESA8.11F*	55°C
			TGACCGCCAGCCTCTTG	*CESA8.11R*	
43	*CESA8.12*	281 bp	TTCGCTTACATCAACACCACCATT	*CESA8.12F*	57°C
			TGCCAACACCACTCCACCTCAT	*CESA8.12R*	
44	*CESA9.01*	241 bp	CCCGCGCCCCCGAGTTCTAC	*CESA9.01F*	60°C
			CACCTTCTGCGCCTTGGACACC	*CESA9.01R*	
45	*CESA9.02*	255 bp	AGCTGCCGCGCCTCGTCTAC	*CSEA9.02F*	61°C
			CCCGGATGGCCTTGTTGTTGTTGA	*CESA9.02R*	
46	*CESA9.03*	303 bp	GTGCAGTTCCCCCAGAGGTTCG	*CESA9.03F*	60°C
			TGGGGCCGCTAGGAGGGTTGTATC	*CESA9.03R*	
47	*CESA9.04*	340 bp	CGACAGGCGCTCTACGGATACA	*CESA9.04F*	59°C
			GACGCCGCCTTCCTCCAT	*CESA9.04R*	
48	*CESA9.05*	222 bp	GCCCCGCCGCTCTCCTCA	*CESA9.05F*	60°C
			CCGCGGCAGTGCATCTTGAACC	*CESA9.05R*	
49	*CESA9.06*	235 bp	GCTTCCGCTGCTCGCCTACTGT	*CESA9.06F*	60°C
			ATGCCCGTCGCGAAGATGGAGA	*CESA9.06R*	
50	*Amy2.01*	233 bp	GGGGAAGAAGAGCCACATTTG	*Amy2.01F*	55°C
			TCGTCGACCTTTCCCAGCATC	*Amy2.01R*	
51	*Amy2.02*	586 bp	GGAAGCAGAGCGGCGGGTGGTA	*Amy2.02F*	60°C
			TCGGCGCAGAGGTGGTTGATGAC	*Amy2.02R*	
52	*Amy2.03*	390 bp	CGTCGGCTGGCATGGTGTTCG	*Amy2.03F*	62°C
			AGCGCTGCGATCTCGTCCTTGAAA	*Amy2.03R*	
53	*Amy2.04*	669 bp	GCTTCTCGCTGCTGCTGCTTCTGT	*Amy2.04F*	60°C
			GATGCCGCGGCTGTCCTTGTAGTC	*Amy2.04R*	
54	*BHM1.01*	558 bp	CGCCGCAGCCTACCCAACAATC	*BHM1.01F*	60°C
			AGCCCAGCCTCACAGCACAGTCG	*BHM1.01R*	

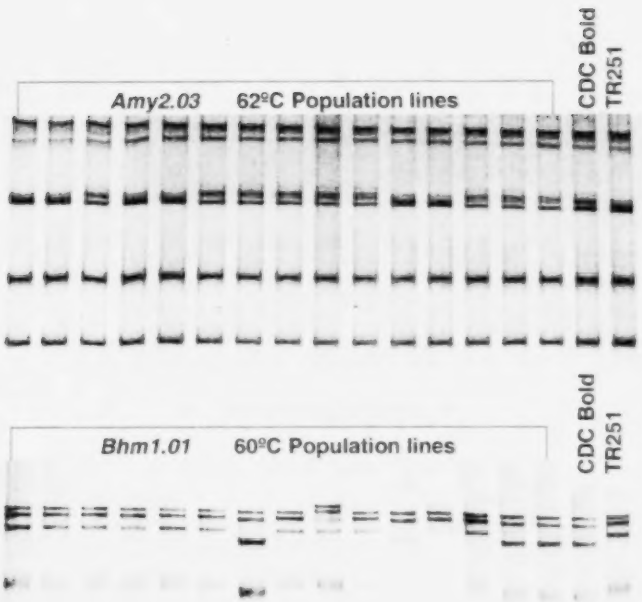


Figure 4. Segregation of two primer pairs *Amy2.03* and *Bhm1.01* in the CDC Bold × TR251 population DH lines on SSCP gels.